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Effects of Dietary Inclusion of Symbiotic on Protein Content, Enzyme Activity and Economic Parameters of *Bombyx mori* L.

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Abstract

Nutrition has a significant importance in improving the growth and development of silkworm *Bombyx mori*. Silk yield is mainly dependent on healthy disease-free larval growth and higher nutritive value of mulberry leaves. Previous studies showed use of synbiotics had higher economic benefits and reduced mortality in silkworm. The present investigation was carried with an objective to determine the impact of fortification of mulberry leaves with Serigrow^{++} (containing *Spirulina*, yeast extract and probiotic bacteria of *Lactobacillus* Spp.) at 1000 ppm on protein content, growth and commercial traits of *B. mori* (CSR2 × CSR4). Feeding of fortified leaves with Serigrow^{++} (1000 ppm) led to increase in 52.73 % protein and 49.7% larval weight over control fifth instar larvae of CSR2 × CSR4 silkworm hybrid. The enzyme activity for protease and amylase was significantly higher in III, IV and V instar in all treated samples in comparison to control samples. Highest protease and amylase activity was observed in Vth instar larva, which was 37.84% and 35.15 % increase over control respectively. Significant increase in various economic parameters like cocoon weight, pupal weight, shell weight and silk filament for treated samples over control were observed. Hence, the results of present study clearly designate the effectiveness of Serigrow^{++} showed better growth, rearing performance, development of silkworm larvae, quality and quantity of silk in *B. mori*.

***Key words*:** *Bombyx mori*, Serigrow^{++}, Synbiotics, Probiotics, Prebiotics, Protein content

Sericulture or farming of silk is a technique which includes the rearing of silkworms for the production of raw silk, out of several commercial species of silkworms, *Bombyx mori* is the used widely and accepted; also intensively studied. In agro-based rural industry for sustainable and integrated farming system sericulture is an important component, with tremendous potential for employment generation and economic returns in rural areas. In India sericulture and allied activities constitute the biggest share in village industry after handloom and khadi, providing full or partial employment to about 6.5 million people [1]. In the world, India stands seconds in total raw silk production next to China. However, the demand for raw silk production is increased than the current production. Silkworms constitute of the larva of a moth (*Bombyx mori*) which is native to Asia that spines a cocoon of fine, strong, lustrous fiber that acts as a source of commercial silk fiber, also it can be easily domesticated. It is a monophagous lepidopteran feeding on mulberry leaves (*Morus alba*). Nutrition (i.e. quality of leaves) plays an important role in overall growth, development and silk production in silkworm, *Bombyx mori* like other organisms. Silkworm is a poikilotherm, it unable to regulate its body temperature and is susceptible to several diseases (viruses, bacteria, fungi, protozoa and similar pathogenic microorganisms) which impact quality and quantity of silk production [2]. In developed countries, the loss due to diseases in sericulture is to the line of 10% of the total crop loss [3], while in developing countries like India the loss is to an extent of 30-40% [4].

Recently nutritional supplements like protein, vitamin, carbohydrates, amino acids, vitamins, hormones and antibiotic etc. have been used in sericulture for better performance of good quality of cocoons [5]. The failure of cellular defense mechanism to control the disease is seen in lower animals as they lack well developed humoral immunity hence vaccine is ineffective in lower animals. The immuno-stimulation can be achieved by use of feed supplement like prebiotics, probiotics and synbiotics as diet supplementation with mulberry leaves which will result in good quality cocoons; thereby improve the quality of silk. Current research has found that probiotics contain live beneficial bacteria play a large role in reducing inflammation, inhibit microbial pathogen growth, food digestion, enzyme secretion and maintain rigidity in the tight junctions between epithelial cells. Today, a mixture of live microorganism (probiotic) and non-digestible oligosaccharides (prebiotic) and together known as synbiotics have been demonstrated to give various benefits stated above. Presently in silk worm diet probiotics like *Lactobacillus* spp., *Strepromyces* spp., *Saccharomyces cerevisiae*, cellulolytic and other beneficial bacterial and prebiotics like *Spirulina*, yeast extract, etc. are used [5-13]. In present study the effect of fortification of mulberry leaves with as synbiotics Serigrow^{++} (containing *Spirulina*, yeast extract and probiotic bacteria of *Lactobacillus*

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Spp.) on enzyme activity and various economical parameters in *Bombyx mori* are seen.

MATERIALS AND METHODS

Insect and synbiotics formulation

The silkworm eggs of *Bombyx mori* belonging to popular Indian bivoltine hybrid (CSR2 × CSR4) procured from government grainage were used in the present study. To attain efficient hatching the eggs were paced in optimum conditions at temperature 25± 2 °C and relative humidity of 70 to 80% in incubator. The selected larvae were reared in plastic trays covered in nylon net. The control and synbiotics applied V1 mulberry eaves were fed to silkworm, five feedings/day and maintained up to cocoon stage [11]. The mature larvae from respective treatments and replications were picked and transferred to the bamboo mountage for cocoon spinning. The cocoons were harvested on 5th day after mounting.

Synbiotics formulation and its effect on growth and development

The synbiotics Serrigrow^{++} (containing *Spirulina*, yeast extract and probiotic bacteria of *Lactobacillus* Spp.) was employed in present study. The 1000 ppm solution of Serrigrow^{++} was prepared distilled water. Every time fresh dilution was prepared and sprayed on mulberry leaves and fed to silkworms. For experimentation 100 larvae were selected for treated Synbiotics were imposed and used only starting from IInd instar onwards. The schedule of application was as follows: IInd instar (first day first feed), IIIrd instar, IVth instar and Vth instar, for control treatment distilled water was sprayed.

Ten larvae were randomly picked from each treatment replication wise and their weight was recorded using electronic balance at the end of each instar for the last three instars (IIIrd, IVth and Vth).

Quantitative protein estimation of silkworm

The total protein content for control and treated larvae at IInd, IIIrd, IVth and Vth instar was calculated using method given by Lowry [14]. The concentration of protein was determined by measuring the absorbance at 660 nm to 720 nm using spectrophotometer.

Enzyme assay: Amylase and protease activity in silkworm gut tissue was estimated for IIIrd, IVth and Vth instar. Mid gut tissue was collected for the estimation of amylase and protease activity. The midgut homogenate was prepared in ice cold phosphate buffer solution pH (6.8). For assay the homogenate was centrifuge at 4000 rpm for 30 minutes and supernatant was used as the enzyme source with appropriate dilution. Then 1 ml of 1% starch solution was added to test tubes meant for amylase activity and 1% casein solution was added to test tubes meant for protease activity. The test tubes were incubated at room temperature for 15 minutes. Then 2 ml of DNS reagent and Ninhydrin reagents were added to amylase and protease test tubes respectively. The test tubes were kept for water bath for 30 minutes. After cooling, enzyme activity was measured at 540nm spectrophotometrically.

Amylase activity = (Concentration of product formed × 2) / (Molecular weight of glucose × time of incubation)

Protease activity = (Concentration of product formed × 2) / (Molecular weight of tyrosine × time of incubation)

Economic parameters

Cocoon characters: The mature Vth instar larvae were picked up from rearing tray and kept on Chandrika for spinning the cocoons. The cocoon was harvested after five days of spinning assessment of various cocoon parameters were made as follows.

Cocoon weight: Ten randomly selected cocoons (five male and five female) were taken and weighed using an electronic balance.

Pupal weight: After removing the floss, the cocoon was taken out without causing any damage to them. Then the ten pupae (five male and five female)

Shell weight: Ten shell weight (five male and five female) of the cocoon, after removing the floss and pupa were weighed using an electronic balance.

Shell %: The shell ratio will be calculated using the following formula and expressed in percentage.

Shell % - Shell weight × 100 / cocoon weight.

Sericin and fibroin content

Sericin content (g) = Initial dry weight of the shell – Dry weight of shell after alkali treatment Fibroin content (g) = Dry weight of shell – Sericin content

Statistical analysis

The data were subjected to statistical analysis of variance for identifying significant difference among the treatment using standard methods.

RESULTS AND DISCUSSION

Effect of synbiotics supplementation on growth of B. mori

The effect of synbiotics Serrigrow^{++}was seen on the silkworm eggs of *Bombyx mori* belonging to popular Indian bivoltine hybrid (CSR2 × CSR4). The results revealed that mature larval weight at different instars of silkworm was significantly increased by feeding on mulberry supplemented with synbiotics in comparison to control (Fig 1). Significant increase in weight of larvae was seen at IIIrd, IVth and Vth instar over control. For IIIrd instar larva of treated weight is increase in 26.59% in IV instar larva of treated weight is increases in 27.28 % and for Vth instar larva of treated weight is increases in 31.57% with respect to control (Table 1).

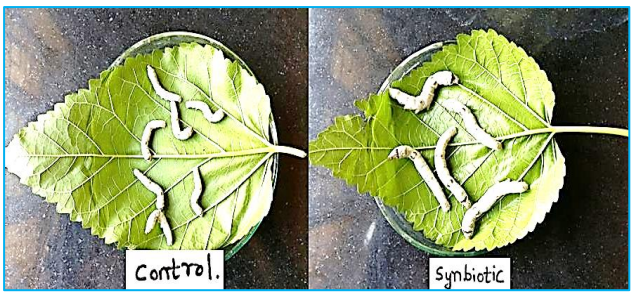

Fig 1 Effect of synbiotics in larval stage of silkworm

Table 1 Effect of synbiotics on larval weight of silkworm

S. No.	Larval weight (mg)			
	Instar	Control (mg)	Treated (mg)	% increase
1	IIIrd	30.28	38.33	26.59
2	IVth	1281.66	1631.33	27.28
3	Vth	3156.72	4153.34	31.57

Protein profile

The silk worm *B. mori* treated with serrigrow++ showed higher protein content than untreated (Table 2). Highest protein content was observed in the Vth instar larva (176.4237).

Table 1 Effect of synbiotics on silkworm protein content

S. No.	Protein (µg per gm tissue)			
	Instar	Control	Treated	% increase
1	IIIrd	102.34	161.88	58.17
2	IVth	94.38	138.98	47.26
3	Vth	115.89	176.42	52.23

Enzyme activity

The silkworm *Bombyx mori* treated with serrigrow ++ showed higher protease and amylase activity in treated than control samples. Highest protease content was observed in Vth instar larva. Protease activity in Vth instar was observed to be 0.074 µmoles/min/ml of sample in control and 0.102 µmoles/min/ml in treated samples; which was 37.84% increase over control. Similarly, the protease activity in IVth and IIIrd was observed to be 0.065 and 0.046 µmoles/min/ml in control and 0.077 and 0.051 µmoles/min/ml respectively. Also, highest amylase activity was observed in Vth instar larva. Amylase activity in tissue was observed to be 0.074 µmoles/min/ml of sample in control and 0.263 µmoles/min/ml in treated samples which was 35.15 % increase over control. Similarly, the protease activity in IVth and IIIrd was observed to be 0.098 and 0.065 µmoles/min/ml in control and 0.156 and 0.072 µmoles/min/ml respectively (Table 3). The increase in the larval and cocoon weight was in correspondence of protease activity measured on Vth instar larvae.

Table 3 Effect of synbiotics on enzyme activity

S. No.	Instar	Amylase enzyme activity (µmoles/min/ml of sample)			Protease enzyme activity (µmoles/min/ml of sample)		
		Control	Treated	% increase	Control	Treated	% increase
1	IIIrd	0.046	0.051	10.87	0.065	0.072	10.77
2	IVth	0.065	0.077	18.46	0.098	0.126	28.57
3	Vth	0.074	0.102	37.84	0.165	0.223	35.15

When synbiotics were fed to silkworm orally through mulberry leaf, it reaches to mid gut and survive for life time and increases the enzyme activity of silkworm and improves its directivity. Similarly, as amylase and protease activity also represented in Table 2, the results indicated that the amylase and protease activity of 5 larvae mainly for the digestion and absorption of sugar and protein content of the mulberry leaves consequently which increases haemolymph and silk gland protein content ultimately increases silk productivity of the silkworm [15]. Glycogen being a storage polysaccharide was found to be high in the experimental groups of silkworm *B. mori*. It is significant to correlate to the availability of increased sugars, which may undergo glycogenesis resulting in of glycogen. Amylase catalyzes the specific hydrolysis of the glycosidic bonds in specific hydrolysis glycosidic bonds in glycogen increased amount of glycogen may the increased secretion of digestive enzyme amylase [16]. Increase in protease activity may be attributed to the increased concentration of silk protein for silk production. The digestive tissue may be tuned to synthesize more of protease enzyme since the protein content increased significantly over control category on UV ray treatment at 280-400 nm [17-18].

Table 4 Effect of synbiotics on various economical parameters

S. No.	Parameters	Control	Treated
1	Cocoon weight (gm)	1.2	1.66
2	Pupa weight (gm)	0.97	1.34
3	Shell weight (gm)	0.97	0.32
4	Shell weight (%)	19.71	18.99
5	Initial dry weight of the shell (gm)	0.23	0.3
6	Dry weight of shell after alkali treatment (gm)	0.191	0.245
7	Sericin content (gm)	0.037	0.054
8	Fibroin content (gm)	0.193	0.245
9	Sericin (%)	16.37	18.25
10	Fibroin (%)	83.62	81.58

Economic parameters cocoon weight (g)

In the treatment effect, cocoon weight was found to be significantly maximum of 1.66 gm for worms fed on mulberry leaves supplemented with Synbiotics (Serrigrow^{++}). Lower cocoon weight was observed in absolute control (1.2gm) (Table 4).

Pupal weight (g)

It can be seen from (Table 3), Worms fed on mulberry leaves supplemented with Synbiotics (serigrow^{++}) concentration recorded significantly maximum pupal weight of 1.342 gm. Lowest pupal weight was recorded in water control (0.97gm).

Shell weight (g)

In case of shell weight (Table 3), shell weight found to be significantly maximum in worms fed on mulberry leaves supplemented with Synbiotics concentration (0.32 gm) and lowest was in absolute control (0.24gm).

Shell ratio (%)

It can be seen from (Table 3) that the shell ratio between the two factors was minimum in treated silkworm (18.99%) and maximum in control silkworm (19.71).

Sericin and fibroin

The silk protein sericin were also increased significantly in the treated worm (18.25). The silk protein fibroin was minimum in treated worm (81.58) (Table 4). Quantitative estimation of enzyme activity of amylase and protease increased treated silkworm set as compare to normal silkworm set.

In the present study, the larval pupal and cocoon length, width and weight were significantly increased in some groups. Many researchers showed that the larval characters improve by different concentration of acid, folic acid, thiamine, vitamin b complex etc., and use these multi-vitamins and minerals compounds could increase the food intake, growth and conversion efficiency of silkworm [12-19]. In the present study, it has been observed that silkworms feed by the particular dose of Serigrow++ have enhanced the larval, cocoon and pupal character were concomitantly increased from 3rd to 5th instars, Also AgNps and *spirulina* which stimulate silkworm to feed more amount of nutrients intake than the control. This

enhancement in larval, cocoon and pupal parameters related to phagostimulation of folic acid [20]. Several authors also reported these effects about ascorbic acid [21]. AgNps attach the surface of pathogenic bacterial cell members and penetrate to release the silver ions and disturb the functions of bacteria [22-23].

CONCLUSION

Nutrition has a significant importance in improving the growth and development of silkworm *Bombyx mori.* Silk yield is mainly dependent on healthy disease-free larval growth and higher nutritive value of mulberry leaves. Previous studies showed use of synbiotics had higher economic benefits and reduced mortality in silkworm. The present investigation was carried with an objective to determine the impact of fortification of mulberry leaves with Serigrow++ (Synbiotic containing algae, fungal extract and probiotic bacteria) at 1000 ppm on protein content, growth and commercial traits of *B. mori* (CSR2 × CSR4). Feeding of fortified leaves with Serigrow++ (1000 ppm) led to increase in 52.73 % protein and 49.7% larval weight over control Vth instar larvae of CSR2 × CSR4 silkworm hybrid. Significant increase in various economic parameters like cocoon weight, pupal weight, shell weight and silk filament for treated samples over control were observed. Hence, the results of present study clearly designate the effectiveness of Serigrow++ showed better growth, rearing performance, development of silkworm larvae, quality and quantity of silk in *B. mori*.

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